

LNP transfection

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 An abbreviated version of this protocol was published in Science Advances in Nov 2020

CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy

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Detailed protocol

LNP transfection-

1. Count live cells using trypan blue manually or by automatic cell counter.
2. Seed 0.1×10^6 cells in tissue culture 12-well plates with 1 ml of growing medium and place for overnight incubation in 37°C, 5% CO₂ incubator.
3. Add LNPs to the growth medium at RNA amounts of 0.1 to 2 µg (Need to be calibrated to each cell line).
 - a. For 005 GBM cells, preincubated LNPs with ApoE3 (0.001 mg/ml) for 20 minutes at 37°C before the addition to the growth medium.
4. Incubate cells for 24 to 120 hours (according to the specific output) in 37°C, 5% CO₂ incubator.
5. Wash cells X 3 with PBS.
6. Collect cells for flow cytometry (72 to 96 hours) or cell cycle assays (24 to 48 hours) using Trypsin-EDTA 0.25%.
7. Wash cells X 3 with PBS.
8. Resuspend cell pellets in Flow cytometry buffer (PBS supplemented with 2% BSA) for flow cytometry or PBS for cell cycle analysis.

Materials:

PBS- Biological industries, Israel.

BSA- Fraction V, MP biomedical, USA. Cat:0216006980X

Recombinant Human ApoE3- PeproTech, USA. Cat:350-02

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Peer, D. , Gutkin, A. and Rosenblum, D. (2020). LNP transfection. Bio-protocol Preprint. bio-protocol.org/prep689.
2. Rosenblum, D., Gutkin, A., Kedmi, R., Ramishetti, S., Veiga, N., Jacobi, A. M., Schubert, M. S., Friedmann-Morvinski, D., Cohen, Z. R., Behlke, M. A., Lieberman, J. and Peer, D. (2020). CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. Science Advances 6(47). DOI: [10.1126/sciadv.abc9450](https://doi.org/10.1126/sciadv.abc9450)

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